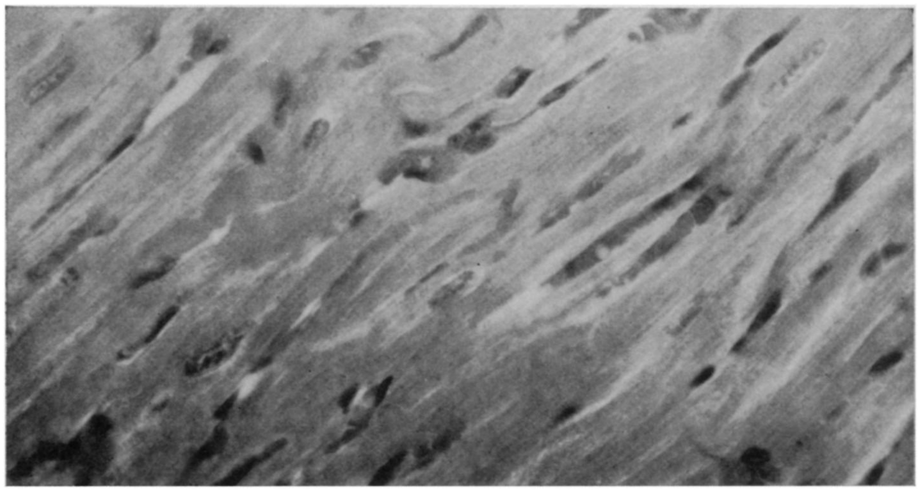
BIOCHEMISTRY

Second hands for biological clocks

Discovery of rhythms regulated by an enzyme system will help pinpoint the time piece at the heart of the body's biological clocks



USDA

Normal rat heart tissue as used in studies of enzymatic oscillators.

Man, contrary to his fondest dreams, was not meant to fly—at least not through several time zones in a matter of hours or out in space where day and night have little relevance.

When he does, the synchronized biological clocks that keep his system moving with a steady, daily rhythm are jarred out of phase.

Indeed, scientists know that persons flying from New York to Tokyo usually need four to five days to recover from initial feelings of fatigue. And they also know that man's susceptibility to both disease and to treatment varies with the clock.

What they do not know is where these biological clocks—common to man and animal alike—are located or what makes them tick.

They know the body temperature rises predictably every morning and drops in the evening. They know the level of hormones in the blood fluctuates according to a regular pattern. They know drugs such as amphetamine are more potent at some hours than at others. But they do not know why.

Recently, however, Pennsylvania scientists identified rhythms for the first time at the biochemical level, discovering what they think may be a model or perhaps even the cellular mechanism that keeps the body's biological rhythms in step. At the Johnson Research Foundation of the University of Pennsylvania, a research team led by Dr. Britton Chance pinpointed an enzyme called phosphofructokinase as the time piece that orchestrates rhythmical activity within individual cells.

The enzyme, found in practically every living cell, is responsible for the biochemical breakdown of glucose into its various end products. Phosphofructokinase, they observed, is the oscillator that controls the fluctuating concentrations of cellular chemicals in intact cells in yeast and in extracts from rats' hearts. The enzyme is unusual because it acts to speed cellular reactions as more and more end products are produced from biochemical reactions. The action of most enzymes is slowed by a negative feedback system; that is, when end products accumulate, they in turn inhibit the enzyme's activity. Phosphofructokinase, on the other hand, operates with increasing speed until all of its substrate, a chemical called fructose 6 phosphate, is depleted from the cell. Then, activity slows down until new quantities of the substrate are carried into cells from the medium.

The main challenge the investigators had to meet in order to make this discovery was in finding a way of observing and measuring fluctuations of chemical concentrations in systems as minute as a single cell. Most readouts of the biochemical properties of living systems come from destructive methods which usually do not preserve the proportions of the system after the cellular destruction occurs. In other words, cells are broken, their components extracted and measured. This method yields concentrations, but not rhythms.

Dr. Chance, and his colleagues, Drs. Kendall Pye and Joseph Higgins, measured a substance called diphosphopyridine nucleotide or DPNH, an intermediary in the breakdown process, is highly fluorescent. When irradiated with ultraviolet light, DPNH gives off a visible blue light. A highly sensitive microfluorometer, used to measure fluorescence, can detect as few as 10,000 molecules of DPNH—one-twentieth the number of molecules in a single mitochondrion, the smallest unit of energy generation in a cell. Rhythmic variations in the intensity of blue light occurred about every 40 seconds.

After observing rhythmic oscillations in single cells, they successfully broke the cell wall and extracted the oscillator without destroying its oscillating ability.

At a convention last month of the Institute of Electrical and Electronics Engineers, Dr. Chance compared the enzymatic oscillators to the electronic oscillators with which engineers are familiar. In general, the oscillations tend to follow the form of a sine wave, he says. However, by altering the chemical composition of the enzyme system, other wave shapes—squares and shoulders, for instance—can be produced. So now, scientists know what chemical reactions cause specific wave shapes or patterns of rhythmic activity.

"We've observed a biochemical oscillation at the point where physiological rhythms ought to originate," says Dr. Pye. "It is possible that now the chemical basis of physiological rhythms will be found."

The potential implications of this work for medicine are vast. Space scientists sending astronauts into orbit need to be able to predict what alterations in biological rhythms will occur and how to deal with them. Physicians administering drugs need to know at what times of day drugs exert more influence than others.

In Addison's disease, for example, doctors want to correct inadequate output of hormones from the adrenal glands. Hormone injections help, but victims seldom feel completely well. According to Dr. Richard Wurtman of the Massachusetts Institute of Technology, one reason for this is that scientists cannot yet exactly duplicate nature's rhythmical pattern of highs and lows of hormone concentrations. However, recent research reveals hormone levels in the blood are highest around 6 a.m. and lowest about 10 p.m. Patients with usually fatal Addison's disease have progressive anemia, low blood pressure, diarrhea and digestive disorders. A number of mental disorders are also known to operate in predictable, rhythmical ways. In some patients, states of hyperactivity are followed with almost complete withdrawal in peaks and valleys occurring about every 48 hours.

In studies with rats, Dr. Lawrence E. Scheving of the Chicago Medical School found that doses of nicotine or amphetamine are fatal at certain times of day but virtually harmless at others. As many as 78 percent of a group of rats died when the drugs were administered at one time of day, but at other hours, the same doses killed only six percent of the animals.

In further studies the Pennsylvania scientists plan to explore the possible relationship between short enzymatic oscillations and those of other biological materials that exhibit natural rhythmicity, such as the 72-per-minute beat of a single heart cell. They also hope to isolate and study the biochemical activity of enzymatic oscillators of longer periods. It is conceivable, they say, that interactions of several enzymatic oscillations may reveal what amount to 24 hour rhythms in series of enzyme systems.

Once scientists gain a real understanding of how biological clocks work, there may be a major revolution in medicine, Dr. Chance says.

If the enzyme oscillator turns out to be the pendulum for the body's 24 hour clocks, that revolution may not be far away. *Tom W. Hill*

POLITICAL SCIENCE

Violence studies frustrating

Political scientists struggle with the causes of violence, but come up with no more firm answers than do amateurs

by Carl Behrens

In the aftermath of a violent summer, and in somber anticipation of another, political violence is absorbing more and more of the attention of social scientists.

Society naturally, if unfairly, looks to the sciences whose laboratory is society itself for answers to its problems. But, as became clear at a recent American Political Science Association conference in Chicago, these scientists are developing more questions than answers.

Though, in those sessions, a dozen papers were concerned with violence directly and many more treated the question in passing, the exercise made clear how inconclusive and fragmentary research in the social sciences can be. It also illustrated the difficulties involved in increasing the responsiveness of the social disciplines to the problems of the real world.

Perhaps the most popular current theory on the cause of political violence is the frustration-aggression process—the idea that rising expectations lead to inevitable frustration, which makes people aggressive and leads to violence. The migration of Negroes from the rural South to urban North in recent decades, for example, is said to have led to unfulfilled expectations, frustration and violence.

Having once stated this premise—which is largely accepted in liberal political circles—the political scientist is faced with the impossible task of defending it. Criticism is leveled on two fronts: the theory is useless because it is too general, and it is inaccurate because it leaves out too many factors.

To be useful, a theory should explain why some outbreaks of violence occurred, and predict where and when future outbreaks will happen. But there is no indication of how much frustration has to be present, or any really effective way of measuring the frustration-level in enough places and times to be significant. So the theory is not very useful in predicting or explaining, however valuable it might be to the fight for more anti-poverty funds.

Moreover, where it can be tested, the theory just doesn't hold up as a single-cause explanation. Dr. Lloyd Free, director of the Institute for International Social Research, has described results of polls he took in Cuba, Nigeria, the U.S. and other violence-prone countries which he says give indications of the frustration level at crucial times before, during and after social upheavals. Some of the experiments support the theory; others don't.

- In Cuba just after the Castro revolution, he found frustration low, which he says explains why the Bay of Pigs invasion didn't get any popular support. And in the Dominican Republic, a 1962 survey showed very high frustration, leading three years later to civil war that resulted in U.S. intervention.
- A poll in Nigeria indicated low frustration, just before the recent outbreak of civil war in that country.
- In the U.S., a 1959 poll of Negroes showed frustration high, although no riots followed; and in 1964, in the midst of riots, frustration was judged considerably lower.

Other analysts point to a number of other factors which help determine where, and when, violence breaks out.

One is unpredictable—some violence-provoking action, perhaps by the police, or knowledge of violence elsewhere, the presence of some target for violence, and curiously, the simple presence of weapons.

The latter was proved in an experiment with college students. Tormented by the experimenter, the students were allowed to vent their aggression on him by giving him electric shocks. Dr. Leonard Berkowitz of the University of Wisconsin found that when a pistol and a rifle were simply in view the reactions were much more violent.

Theories of violence abound, but scientific data about them are lacking. Is the man who kicks his cat less likely to punch his boss in the nose? Some social scientists say yes, but others hold that violence, even against safe targets, can lead to more violence.

The violence brought to every living room via television may purge viewers of violent tendencies, or inflame them. No one knows. Both views are held.

There are political factors as well: the legitimacy of the government, opportunities for political expression, the amount of communication between government and governed.

One theory often expressed is that violence may spring from rebellion against an inhuman, push-button, automated, mechanized society. Another, that society worships violence. Dr. Joost A. M. Meerloo of the New York School of Psychiatry calls this effect, "the holiness of hostility."

This list is not complete and there is no way to rank such factors in order of importance. There is even a theory that meat eaters are more violent than persons who get their proteins from beans —but it has never been tested.